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FOREST INSECT INVESTIGATIONS

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FOREST INSECT SURVEY

NORTH COAST SUBREGION OF CALIFORNIA

Studies of Resistance of Pine Hybrids

to Insect Attack

at

Institute of Forest Genetics

Seasons of 1949 and 1950

by

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Studies of Resistance of Pine Hybrids to Insect Attack at Institute of Forest Genetics Seasons of 1949 and 1950

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STUDIES OF RESISTENCE OF PINE HYBRIDS TO INSECT ATTACK

Institute of Forest Genetics - Seasons of 1949 and 1950

Studies of resistance to the pine reproduction weevil were carried on during the seasons of 1946 - 1948 inclusive, using cage controls to test a number of young-aged trees. This work was summarized at the close of the 1948 season with results that indicated a highly variable degree of resistance between pine species, hybrids, and individual trees. This study has been reported upon in Forest Research Note No. 68, issued in April 1950.

Further studies of resistance to weevil attack were carried on during the seasons of 1949 and 1950 which have not been reported upon as yet. In addition to weevil studies, other work was initiated during the 1950 season to determine the potential resistance of new hybrids to attack by Dendroctonus and other bark beetles. This report is intended to put on record the procedures and results of these more recent studies.

I. Pine Reproduction Weevil Studies - Season of 1949-50.

1. Further cage control tests with the Jeffrey-Coulter hybrid, 1949.

A. Conditions of the 1949 experiment

The tests that were made in previous years were limited to small cage controls on 4- to 7-year-old trees still growing in the original nursery beds. These cages were large enough to enclose only from 10 to 20 trees representing one or at most two varieties of pines. The weevils were therefore quite restricted as to choice of the strains which they could attack. The adults were largely forced to attack trees that had been selected for them. While this method served to demonstrate any inherent resistance on the part of the caged trees, it left unanswered the question whether or not the weevils would have attacked these trees if they had been given a free choice under natural conditions.

For the ~~1950~~ 1949 season an effort was made to give more latitude for selection of host trees by weevils in the cage. In order to do this it was necessary to construct a much larger cage than any used previously.

B. Type of cage used in test

One of the beds in the 1946 nursery was selected as the site for the 1949 test. This bed contained parallel rows of Jeffrey pine, Coulter pine, and the Jeffrey-Coulter hybrid in their fourth year of growth. Cage construction consisted of a wooden frame, 6 feet wide, 12 feet long, and 7 feet high covered with 32 mesh plastic screen.

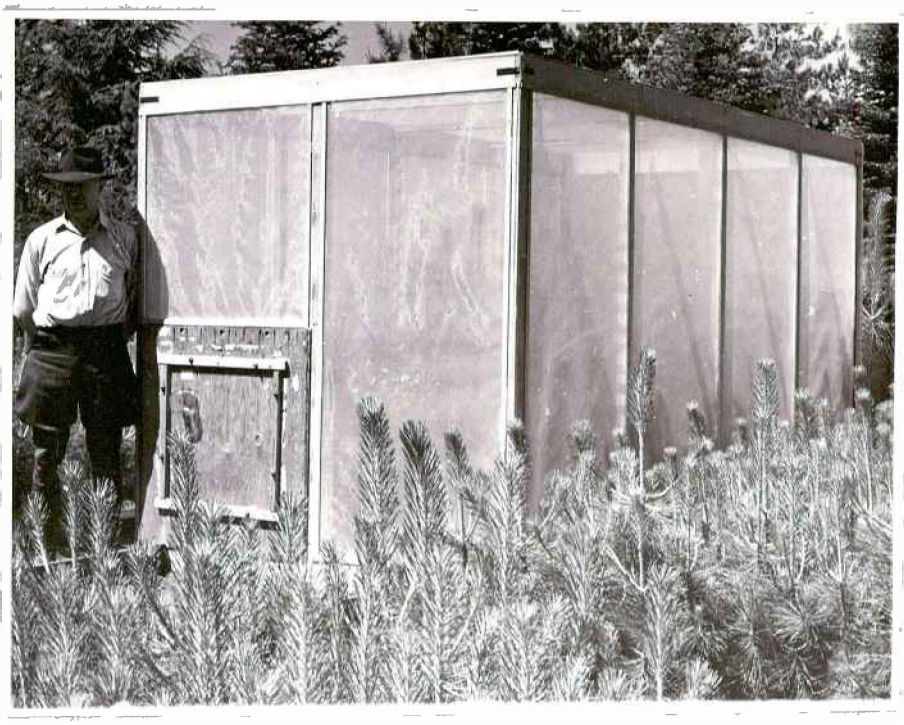


Figure 1

The number of trees enclosed within this structure were:

Jeffrey pine - - - - - - - - - - - - - - - -	50
Coulter pine - - - - - - - - - - - - - - - -	30
Jeffrey-Coulter hybrid - - - - - - - - - -	44
Total	124

C. Source of weevil population used in test

One of the chief objectives of the procedures used in this test was to stock the cage with a very heavy adult weevil population, sufficient in numbers to attack and kill all enclosed trees if susceptible. To insure an abundance of new adults, overwintering larval broods were collected from several sources:

	Trees
1. Infested ponderosa and scopulorum pines, 4 - 7 years old which were attacked during the 1948 season in nursery - -	160
2. Jeffrey pines from Burney brushfields - - - - - - - - - -	15
3. Jeffrey pines, 10 years old from progeny outplantings- - -	2
Total	167

D. Rearing and liberation procedures

In the experiments prior to this season adult weevils were obtained by keeping the infested stems in cans until the adults emerged. The adults then were kept in the emergence jars until they could be transferred to the test cages. In the 1949 experiment the infested stems were held in rearing cans during the early emergence period of the parasites and until the first adult weevils appeared. As soon as the adults started to emerge, the infested stems were placed in the cage, mainly on the ground among the enclosed trees. Here the adults emerged along with the parasites that still remained in the material, and were free to attack the enclosed trees without further handling. This procedure did away with the tedious rearing, screening, and liberation of adults in the cages. It also permitted conditions for the adults which approximated as nearly as possible those in the field.

E. Seasonal developments within the cage

Following are the summarized notes in chronological sequence:

1949

May 18 — Cage installed in nursery bed.

May 25 — Infested stems placed in cage.

June 10 — Many new adult weevils crawling about on walls of cage. (Emergence started several days prior to this date).

June 15 — Adults feeding on needles. Fewer weevils are on cage walls. Many adults have not yet emerged from infested stems.

June 20 — First attacks noted on stems of live trees.

July 6 — Emergence completed--estimated that about 9000 weevils had emerged in cage. Attacks heavy on stems of all living trees with no noticeable preference between Jeffrey, Coulter, and hybrid trees.

August 30 — Adult weevils still active on stems and on walls of cage. New attacks still in progress. No trees have faded up to this date.

(Because the rapid growth of the Coulter pines was overcrowding and shading the Jeffrey and hybrid trees, 19 of the Coulter pines were cut and removed in order to permit better light and air conditions in the cage.)

September 12 — Activity of the adults has largely subsided. All trees in the cage show heavy attack on the stems and a number of the Jeffrey pines have faded.

F. Tree mortality at end of test

An inventory of dead and living trees in the cage was taken in November 1949 and again in April 1950, when no change was found from the mortality in the preceding fall. This is shown in Table 1 and in Figure 2.

Table 1.--Mortality of Pinus jeffreyi, P. coulteri, and P. jeffreyi x (P. j. x P. c.) from weevil attack under cage control, season of 1949.

Species	Number of trees			Mortality
	Living	Dead	Total	Percent
Pinus jeffreyi	36	14	50	20.8
Pinus coulteri	1/ 30	0	30	0
P. jeffreyi x (P. j. x P. c.)	43	1	44	2.3

1/ This figure includes 19 trees cut and removed August 30. These trees were considered to be resistant when cut. This group will be discussed later.

Mortality of the Jeffrey-Coulter hybrid. One of the hybrids was counted as weevil kill because it contained successful larval development. This tree was heavily suppressed by the more vigorous Coulter pines and was obviously making little or no growth. It was demonstrated in previous studies that when trees of this hybrid are infested by the egg and young larval stages of the weevil, development of the insect is inhibited as long as the tree maintains its normal growth functions; however, if the growth is stopped by cutting off the tree at the root level, the larval broods begin to grow rapidly and complete their development. All other hybrid trees in the cage successfully resisted attack in 1949 and made normal growth in 1950. The results of this test serve to confirm those of previous years, i.e., that the Jeffrey-Coulter hybrid resists weevil attack as long as the tree maintains transpiration and other vital physiological functions. Only when this hybrid ceases to maintain normal functions of growth does it become a suitable host for the weevil.

Development of weevil larvae in Coulter pines. All of the Coulter pines in the cage were heavily attacked and produced strong pitch flow on the stems. None, however, showed any evidence of succumbing up to the end of August when 19 of the trees were cut to provide better light and air conditions in the cage. One purpose served by cutting these Coulter trees was to determine whether the weevil larvae could complete their development as they do in hybrids when the trees are cut. This point was first tested in 1948 when 3 infested Coulter pines were cut in September. It was found then that the larvae carried their development somewhat further than in the living trees, but all died eventually before they were full-grown.

Similar results were found in the infested Coulter trees cut August 30, 1949. In December it was found that a few larvae had extended mines as deep as the cambial area, and in some cases had started mines into the sapwood; however, these larvae were undersized and later examinations indicated that they failed to pupate. These results were fairly positive in demonstrating that Coulter pine is not an acceptable host tree for the weevil even after the vital living functions of the tree have broken down.

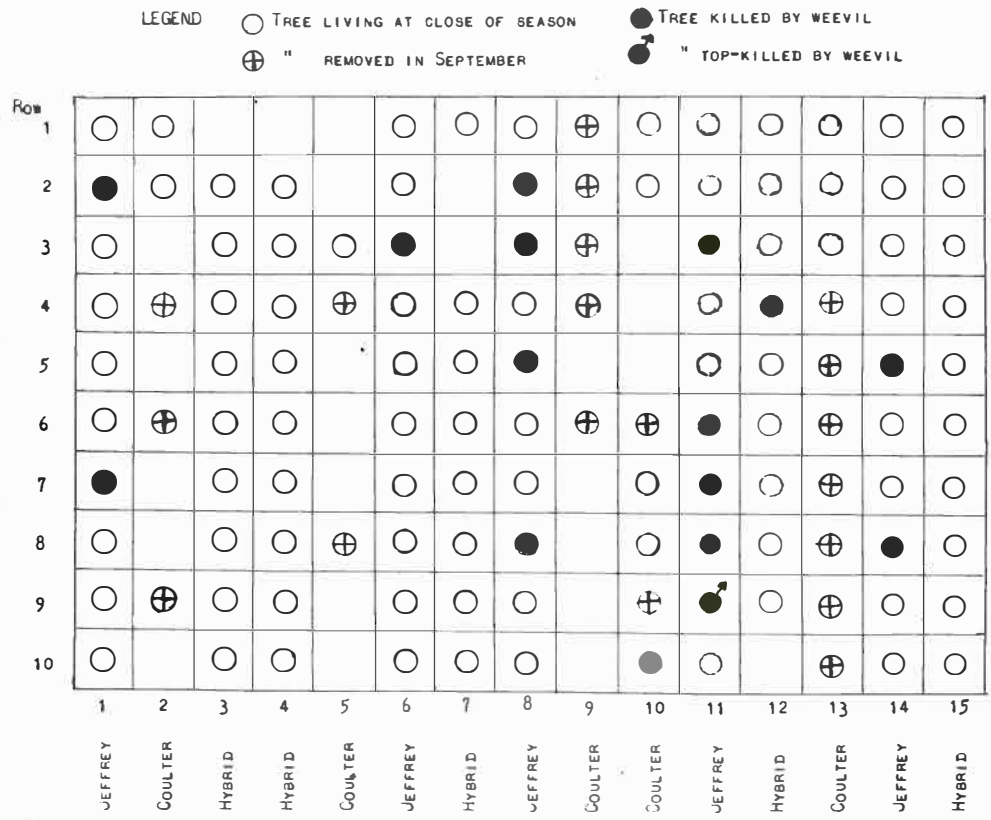

LEGEND
TREE LIVING AT CLOSE OF SEASON
" REMOVED IN SEPTEMBER
TREE KILLED BY WEEVIL
" TOP-KILLED BY WEEVIL
Row
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
JEFFREY COULTER HYBRID HYBRID COULTER JEFFREY HYBRID JEFFREY COULTER COULTER JEFFREY HYBRID COULTER JEFFREY HYBRID

Figure 2

Chart showing location of Jeffrey pines, Coulter pines, and the Jeffrey-Coulter hybrids in cage and mortality during season of ~~1950~~ 1949, due to weevil attack.

2. Weevil selection of ponderosa pine strains without cage controls.

In the 1946 nursery planting at the Institute, 7 beds were planted so as to obtain a comparison of the early growth behavior of 8 regional strains of ponderosa pine including forms from British Columbia, eastern Washington, Montana, eastern and western Oregon, and California strains found in Eldorado and Santa Cruz Counties. Included in the planting were a number of crosses from pollen of each of these regional strains with two cone-bearing Eldorado trees. For the 1949 season all of these trees were in their fourth year of growth and were becoming badly overcrowded in the nursery beds.

During the late summer of 1949 weevil attacks were noted in these nursery beds and by fall a number of the trees were dead. It was decided then to let the weevil infestation run its course for the next two seasons to see if there was a trend toward selection of some strains over others. A protective spray program was initiated on valuable trees in the nursery and outplantings of the Institute to protect them against the weevil.

After the new adult weevils had emerged in the spring of 1950 an inventory was taken of the nursery beds, and the mortality percent determined for each strain and cross represented. The results of this first inventory are shown in Table 2.

Another inventory will be necessary in the summer of 1951 after emergence from the 1950 attack is completed. The 1950 attacks were fairly heavy and these, added to the results of the 1949 attack, should show whether or not there is marked preference for any of the strains. Results of the 1949 attack are inadequate for conclusions but they show a slight trend toward selection of the Montana and eastern Washington forms and for hybrids in which these strains are present.

Table 2.--Mortality caused by Cylindrocopturus eatoni in strains and hybrids of ponderosa pine, season of 1949.

1946 Nursery - Institute of Forest Genetics

Experiments: 21-32 -- Hybrids vs. parent strains

Experiment number		Living	Suppressed	Weevil kill	Top kill	Total	Weevil kill Percent	Top kill Percent
	Wind							
21	P-Eld-4B x 56 x wind	78	2	19		99	19	
25	"	59		15	3	77	19	4
26	"	97		2		99	2	
27	"	88		7		95	7	
28	"	89		8	3	100	8	3
29	"	94	1	1		96	1	
23	PP-Chelan N. F.	60	3	23	2	88	26	2
32	"	80		5		85	6	
31	PP-Ore Mix	61				61		
24	PP-Bitterroot	5	3	12	2	22	55	9
	Total	711	9	92	10	822		
	Hybrid							
27	P-Eld-4Bx56xPP-Ore-Crn	72		8		80	10	
29	P-Eld-4Bx56xPP-Ore-Jos	92	1	3		96	3	
31	P-Eld-15-43xPP-Ore-Jos	97				97		
24	P-Eld-4B-56xPP-Mont	77	2	12	3	94	13	3
25	P-Eld-4B-56xPP-Mont	37		27		64	42	
21	P-Eld-4B-56xPP-Wash	67	3	29		99	29	
23	P-Eld-4B-56xPP-Wash	65		19		84	23	
32	P-Eld-15-43xPP-Wash	63		1		64	2	
28	P-Eld-4B-56xPP-Br.Col.	78	2	15	4	99	15	4
26	P-Eld-4B-56xPP-Pac.Coast	90	2	5	1	98	5	1
	Total	738	10	119	8	875		

Table 3.--Hybrids of P. ponderosa-Eld. with pollen from 8 different sources (Latin squares)

Experiments: 22 and 30

Hybrid	Living	Suppressed	Weevil kill	Top kill	Total	Weevil kill	Top kill
						Percent	Percent
Experiment 22. P-Eld-4B-56 x pollen from 8 sources							
PP-Wash	22		16		38	42	
PP-Mont	29		11		40	28	
PP-Br. Col.	29	1	10		40	25	
Wind	29		10		39	26	
PP-Ore-Cra	28		10	2	40	25	5
PP-Bitterroot	29		11		40	28	
PP-Pac. Coast	29		11		40	28	
PP-Ore-Jos	27	1	12		40	30	
Total	222	2	91	2	317		
Experiment 30. P-Eld-15-43 x pollen from 5 sources							
Mont.	39				39		
Ore-Jos	39				39		
Wash	32	1		1	34	3	3
Br. Col.	38	1	1		40	3	
Ore-Cra	39				39		
Total	187	2	1	1	191		

II. Bark Beetle Studies - Season of 1950.

1. Conditions limiting bark beetle tests.

The decision to initiate preliminary studies of resistance of new pine hybrids to bark-beetle attack was reached between representatives of the Forest Insect Laboratory and the Institute of Forest Genetics at a conference held in March 1950. Since most of the hybrids now growing at the Institute are of recent origin, the trees are too young to be suitable for bark-beetle attack. They will have to reach the "black-jack stage" when the thick outer bark plates have formed, before Dendroctonus beetles can successfully attack and develop broods in them. Many of the contemplated tests with Dendroctonus and Ips beetles will have to wait some 15 to 20 years hence. However, definite plans are needed now in order that planted trees can be made available and the necessary facilities arranged for conducting the experiments when the time arrives.

2. Potential role of the turpentines.

In the meantime some preliminary tests can be made which will serve the purpose of developing techniques and exploring the basic factors involved in tree resistance to bark-beetle attack. One of the most promising leads for study is the role of turpentines in protecting pines against invasion by insects that feed in the inner bark and cambium. This factor was brought out into the open by results of the weevil studies when it was found that turpentine production was stimulated by the attack, and that the transfer of these turpentines to the phloem and cortex apparently served to inhibit growth of the larvae. In resistant trees complete mortality of the larvae eventually resulted.

Similar processes on the part of the tree may very well operate successfully against attack by bark beetles. Unless the beetles, either by special adaptations in their habits or with the aid of associated microorganisms, overcome the toxic effects of turpentine production in their preferred host species, the attack is not successful and the beetles are "pitched out." Some trees are now available at the Institute of Forest Genetics which can be used for preliminary studies to determine whether turpentines are a dominant factor in protection of the tree against bark-beetle attack.

In the first plantings in the Eddy Arboretum a number of trees were planted from seed produced by Jeffrey pines which have now completed their twenty-third year of growth. Bark thickness of the lower trunk is apparently sufficient to permit successful bark-beetle attack. The trees were expected to produce Jeffrey pine characters, but when they reached cone-bearing age, one tree in particular (R70-L32) displayed characters that were intermediate between Jeffrey and ponderosa pines. To obtain further evidence on possible hybridization, Mirov made an analysis in 1949 of the turpentines of this tree by the distillation process. He found that the turpentines included the characteristics of both Jeffrey pine and ponderosa pine, and Mirov therefore considered it to be a true hybrid. Adjacent to

this tree was another (R70-L33) from the same seed source which produced mainly pure heptane and was therefore considered to be a true Jeffrey pine. (See distillation charts, Figure 3)

3. Plan for 1950 test on Jeffrey-ponderosa hybrid and Jeffrey pine.

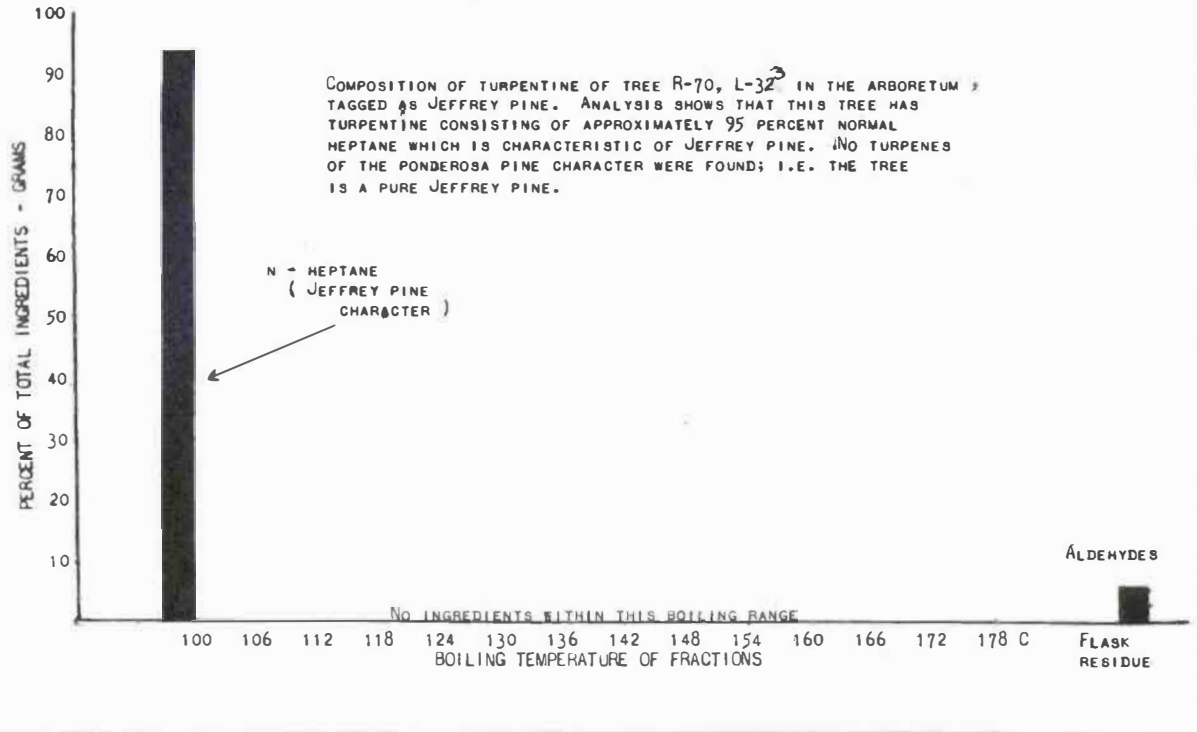
Dr. Palmer Stockwell suggested that the hybrid be tested for resistance to bark beetles that are known to attack Jeffrey and ponderosa pines in nature. Plans were made to test both of these trees by caged attacks of the Jeffrey pine beetle (Dendroctonus jeffreyi) during the season of 1950, using the pure Jeffrey pine as a check against the hybrid. This type of cage was a screen type, enclosing the lower 10 feet of the trunk, and has been used successfully in previous entomological studies to control attack of Dendroctonus beetles on ponderosa and sugar pines. (See Figure 4)

The living Jeffrey beetle adults used for the test were secured by rearings from 2 logs cut from an infested tree in Lassen National Park. The rearing cage was set up at the Institute but the logs failed to produce as many adults as expected and only enough were secured to stock the cage enclosing the hybrid. The western pine beetle, which was available in summer generation broods in ponderosa pines near the Institute, was substituted for the Jeffrey beetle on Jeffrey pine, mainly to see if the western pine beetle could be forced to attack a tree containing the heptane characters of Jeffrey pine. Condensed notes of the experiments on each tree follow.

4. Dendroctonus jeffreyi vs. Jeffrey-ponderosa hybrid (Tree R70-L3

1950

June 15	Cage installed on tree.
June 29 - July 18	Adult Jeffrey beetles emerging from rearing cage at rate of 2 to 11 per day, were liberated in tree cage. During this period a total of about 200 beetles were placed in the cage.
July 13	First pitch tubes noted on outer bark of enclosed tree.
August 14	Cage removed--counted 52 pitch tubes on bark surface which had been enclosed by cage. No living beetles were found--several dead beetles found imbeddedin pitch flow.
October 18	It is obvious now that all attacks have been "pitched out." The beetles attacking the tree apparently penetrated as far as the cambium, but lack of any sawdust in the pitch flow indicates that no egg galleries were constructed. (See Figure 5)


COMPOSITION OF TURPENTINE OF TREE R-70, L-32 IN THE ARBORETUM, TAGGED AS JEFFREY PINE. ANALYSIS SHOWS THAT THIS TREE HAS TURPENTINE CONSISTING OF APPROXIMATELY 95 PERCENT NORMAL HEPTANE WHICH IS CHARACTERISTIC OF JEFFREY PINE. NO TURPENES OF THE PONDEROSA PINE CHARACTER WERE FOUND; I.E. THE TREE IS A PURE JEFFREY PINE.
N - HEPTANE
(JEFFREY PINE CHARACTER)
PERCENT OF TOTAL INGREDIENTS - GRAMS
100
90
80
70
60
50
40
30
20
10
ALDEHYDES
NO INGREDIENTS WITHIN THIS BOILING RANGE
100 106 112 118 124 130 136 142 148 154 160 166 172 178 C
FLASK RESIDUE
BOILING TEMPERATURE OF FRACTIONS

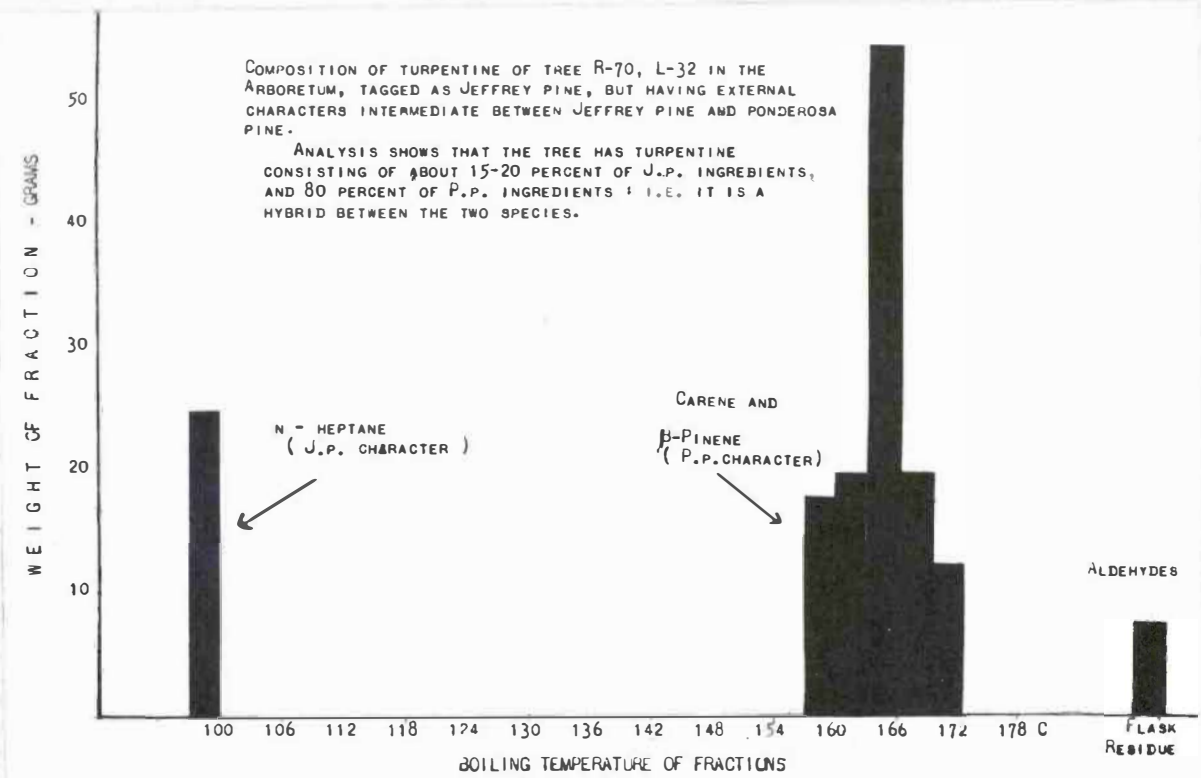

COMPOSITION OF TURPENTINE OF TREE R-70, L-32 IN THE ARBORETUM, TAGGED AS JEFFREY PINE, BUT HAVING EXTERNAL CHARACTERS INTERMEDIATE BETWEEN JEFFREY PINE AND PONDEROSA PINE.
ANALYSIS SHOWS THAT THE TREE HAS TURPENTINE CONSISTING OF ABOUT 15-20 PERCENT OF J.P. INGREDIENTS, AND 80 PERCENT OF P.P. INGREDIENTS ; I.E. IT IS A HYBRID BETWEEN THE TWO SPECIES.
WEIGHT OF FRACTION - GRAMS
50
40
30
20
10
N - HEPTANE
(J.P. CHARACTER)
CARENE AND
B-PINENE
(P.P. CHARACTER)
ALDEHYDES
100 106 112 118 124 130 136 142 148 154 160 166 172 178 C
FLASK RESIDUE
BOILING TEMPERATURE OF FRACTIONS

Figure 3

ANALYSIS OF THE TURPENTINES OF 2 PINES CAGED FOR BARK-BEETLE ATTACK
Institute of Forest Genetics - Season of 1950

Tree R-70, Line 33, a pure Jeffrey pine, was subjected to attack by the western pine beetle.

Tree R-70, Line 32, a hybrid of Jeffrey and ponderosa pines, was subjected to attack by the Jeffrey pine beetle.

In neither tree was the attack successful.

Figure 4

CAGE USED FOR FORCING ATTACK OF JEFFREY PINE BEETLE ON A HYBRID OF JEFFREY AND COULTER PINES.

The adult beetles were obtained from infested Jeffrey pine logs kept in a separate rearing cage. They were collected in a mason jar which was then screwed into the panel on north side of cage enclosing tree. From the jar the beetles were free to enter cage and attack the enclosed tree.

Cage was kept on tree during period of the attack, then removed.

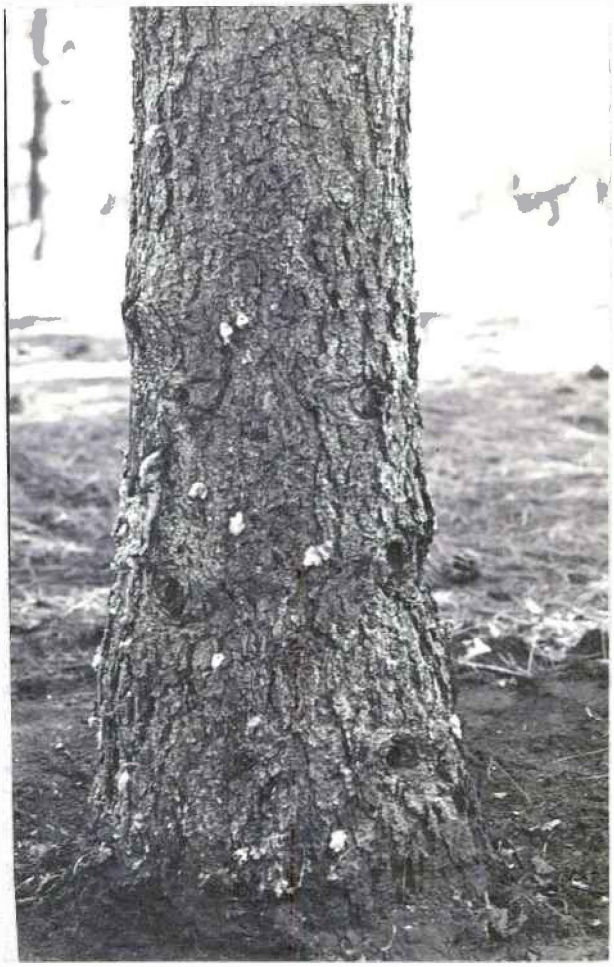


Figure 5

THE SAME TREE AS IN FIGURE 4 AFTER CAGE WAS REMOVED IN AUGUST. THE WHITE SPOTS ARE PITCH TUBES FORMED BY RESIN FLOW AT PLACES WHERE BEETLES BORED THROUGH THE OUTER BARK AND PHLOEM.

When cage was removed, 52 pitch tubes were counted on enclosed area. Most of these attacks were on the lower part of the trunk. It was found that the beetles penetrated no further than the cambium and were eventually overcome by the flow of turpentine produced by the tree. A number of beetles were found dead in the pitch masses.

No egg galleries were made by the beetles and by September it was obvious that the tree had successfully resisted the attack.

5. Dendroctonus brevicomis vs. Jeffrey pine (Tree R70-L33)

1950

June 15	Cage installed on tree.
August 15 - 16	About 30 square feet of ponderosa pine bark containing full-grown larvae and pupae of the western pine beetle placed in cage, so that the new adults would emerge in cage enclosing tree.
September 13	Emergence from infested pine bark has been completed. Bark was removed from cage and trunk of tree examined for attacks. High temperatures preceding and during emergence period had apparently caused high mortality of western pine beetle larvae and pupae, but a count of exit holes indicated that at least 400 beetles had emerged in cage between August 15 and September 13. No pitch tubes or other evidence of attack on tree could be found.

III. Summary of Experiments and Studies, 1949-50.

1. Resistance of Jeffrey-Coulter hybrid to weevil attack.

Further tests in 1949 comparing resistance of Jeffrey and Coulter pines and the Jeffrey-Coulter hybrid were based on a larger number of trees than in previous experiments. Mortality from weevil attack for the 1949 test averaged:

Coulter pine - - - - - - - - -	0 percent
Jeffrey-Coulter hybrid - - -	2.3 percent
Jeffrey pine - - - - - - - - -	28.8 percent

Mortality of the hybrid consisted of one badly suppressed tree. All other trees in the test successfully resisted heavy weevil attack. These results, along with those of preceding tests, support the conclusion that,

a. Jeffrey pine is generally susceptible;

b. Coulter pine is inherently resistant;

c. The Jeffrey-Coulter hybrid is resistant as long as normal functions of the tree are maintained. The weevil can attack successfully only when tree metabolism is stopped suddenly, or becomes greatly slowed down.

2. Selection of ponderosa pine strains by weevil.

Among a series of regional strains of ponderosa pine exposed to uncontrolled attack by the weevil, some preference was shown for Rocky Mountain forms. This study will have to be continued for another year or two before conclusions are warranted.

3. Preliminary studies of resistance to bark-beetle attack.

Cage-controlled experiments indicated that the Jeffrey-ponderosa hybrid is resistant to attack by the Jeffrey beetle. Chemical analysis of the turpentines of this hybrid gives reason to assume that resistance to bark-beetle attack is tied up in some way with the composition of the turpentines that are produced in the tree. This is a phase of insect resistance studies which deserves further exploration.

Figure 6

Work of Cylindrocopturus eatoni in ponderosa pine reproduction restocking the Harris Ranch Burn, Sierra National Forest. The opening (now taken over by bear clover) was caused by a group killing by the weevil about 10 years before this picture was taken. A few dead trees are still in evidence. Large tree in center survived attack and has now outgrown susceptibility to the weevil.

Figure 7

Out-planting at the Institute of Forest Genetics. At left - Row of Jeffrey-Coulter hybrids, now 10 years old; Center - Two rows of Jeffrey pines, same age; Background - Coulter pine, same age. The hybrids and Coulter pines have reached the size where the weevil will no longer attack them. The Jeffrey pines, because of their much slower growth, are still susceptible and will require a much longer time to attain immunity.